compact fold with transient structural features resembling those seen previously in the X-ray structure of an active complex containing PDEγ46–87. NMR mapping of the interaction between PDEγ46–87 and the chimeric PDE5/6 catalytic domain confirmed that the C-terminal residues (74–87) of PDEγ are involved in the association and demonstrated that its W70 indole group, which is critical for subsequent binding to α_t, is left free. These results indicate that the interaction between PDEγ and α_t, which occurs during the phototransduction cascade, involves the selection of preexisting transient conformations. We compare these results with those from a published NMR study (1) of another intrinsically disordered protein, a plant-specific protein in the photosynthetic thylakoid membrane (TSP9). This study showed that non-phosphorylated TSP9 and a mimic of its tri-phosphorylated form both are disordered under aqueous conditions but adopt an ordered conformation in the presence of detergent micelles. The results provided a structural model for the role played by TSP9 in its biological function.

References

1. Song, J., M. S. Lee, I. Carlberg, A. V. Vener, and J .L. Markley. 2006. Micelle-Induced Folding of Spinach Thylakoid Soluble Phosphoprotein of 9 kDa and Its Functional Implications. Biochemistry *45*:15633–15643.

Platform AH: Self-Assembled Session: KCNE Peptides (MiRPs): Essential Components of Voltage-gated Potassium Channels

1744-Plat KCNQ1 S6 Segment Determines Modulation by KCNE4

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The voltage-gated potassium (Kv) channel KCNQ1 is modulated by members of the KCNE family of single transmembrane proteins. The related Kv channel KCNQ4 can also be modulated by KCNE proteins but with very different effects. For example, KCNE4 completely suppresses current carried by KCNQ1 but not KCNQ4 channels. Using a KCNQ1/KCNQ4 chimera strategy, we have mapped structural determinants of the KCNE4 effect. Based upon previous work, we hypothesized that important structural determinants of KCNE4 interaction reside within the S6 segment. Using heterologous expression in CHO cells we first examined the functional properties of a KCNQ1 chimera having the S6 sequence of KCNQ4 in the absence or presence of KCNE4. Unlike the parent KCNQ1 channel, this chimera was resistant to the suppressive effects of KCNE4. By contrast, the converse chimera (KCNQ4 with the S6 of KCNQ1) was dramatically inhibited by KCNE4. Subsequent KCNQ1 chimeras incorporating subregions of S6 from KCNQ4 also exhibited resistance to KCNE4. However, a KCNQ1 chimera in which four residues in the N-terminal half of S6 were substituted with the corresponding residues of KCNQ4 (Q1–Q4 [324–328]) was resistant to KCNE4 inhibition. Interestingly, this chimera still generated ^{1}Ks when co-expressed with KCNE1. Our results indicated that the KCNQ1 S6 segment contains residues critical to the channel's response to KCNE4 but are independent of structures required for the modulation by KCNE1.

1745-Plat KCNQ1 and KCNE1 Make State-dependent Contacts in Their Extracellular Domains

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Previous studies on (KCN)Q1/(KCN)E1 interactions in the IKs channel complex largely focused on the transmembrane/cytoplasmic regions. However, intimate interactions between Q1/E1 in their extracellular domains are possible: short QT- or familial-atrial-fibrillation (fAF)-related Q1 mutations S140G & V141M (immediately outside S1) do not affect Q1 channel function when expressed alone, but markedly affect the IKs channel function when coexpressed with E1. Likewise, mutations in E1 extracellular domain have been linked to long QT or fAF, indicating perturbations of IKs channel function. We used the 'disulfide bond mapping' approach to explore extracellular contact points between Q1 and E1, focusing on Q1 positions 140–147 and E1 positions 36 – 43. Native Cys(teines) were removed. New Cys were engineered into positions listed above. We expressed different combinations of Cys-substituted Q1/E1 pairs in COS-7 cells and used Western blotting under non-reducing conditions to test which pairs could form disulfide-bonded Q1/E1 complexes (80-kDa, vs Q1 60-kDa and E1 20-kDa). WT-Q1/Cys-substituted E1 or Cys-substituted Q1/WT-E1 served as negative control, and we tested whether DTT could abolish the 80-kDa band. For positive Q1/E1 pairs, we used oocyte expression to check: (a) mutations per se did not disrupt Q1/E1 interactions, and (b) putative disulfide bond formation impacted on IKs channel function. We identified disulfide bond formation between Q1-144C and E1-40C or E1-41C, and between Q1-145C and the same E1 Cys residues. Oocyte experiments suggested that Q1-145C formed disulfide bond with E1-40C preferentially in the open state, and the disulfide-bonded conformation locked the IKs channel in a constitutively active state. In contrast, disulfide bond formation between Q1-I145C and E1-41C stabilized the IKs channel in the closed state. The state-dependent contacts between Q1 & E1 will be useful spatial constraints in building IKs structural models of different gating states.

1746-Plat S1 Constrains S4 Sensor In Kv7.1 K^+ Channels: Interaction With KCNE1 Auxiliary Subunit

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Voltage-gated K^+ channels comprise a central pore enclosed by four voltage-sensing domains (VSD). While movement of the S4 helix is known to couple to channel gate opening and closing, the nature of S4 motion is unclear. Here, we substituted S4 residues of Kv7.1 channels by cysteine and tryptophan and recorded whole-cell mutant channel currents in *Xenopus* oocytes and CHO cells, respectively. In the closed state, disulfide and metal bridges constrain residue S225 (S4) nearby C136 (S1) within the same VSD. In the open state, two neighboring I227 (S4) are constrained at proximity and residue R228 (S4) is confined close to C136 (S1) of an adjacent VSD. KCNE1, which assembles with Kv7.1 to form the cardiac I_{KS} current, disturbs the S4–S1 inter-VSD constraint. Structural modeling predicts that in the closed to open transition, an axial rotation (~190°) and outward translation of S4 (~ 12 Å) is accompanied by VSD rocking. This large sensor motion changes the intra-VSD S1–S4 interaction to an inter-VSD S1–S4 interaction. These constraints provide a ground for cooperative subunit interactions. They also suggest a strategic location of KCNE1 wedged close to S1 and S4 of two adjacent VSDs and nearby S6, thereby accounting for its dramatic impact on Kv7.1 gating and permeation.

1747-Plat Stoichiometric Determination of Membrane-Embedded β-subunits in Functioning K^+ Channel Complexes

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Voltage-gated KCNQ1 K^+ channels are tetrameric integral membrane proteins that open and close in response to changes in membrane potential. Different cell types co-assemble ion conducting KCNQ1 α-subunits with different regulatory KCNE β-subunits to produce physiologically relevant complexes with diverse ion conducting and gating properties. Determining the β-subunit stoichiometry of these membrane protein complexes has been challenging and fraught with contradictory results, and thus the number of partnering peptides has not been definitively determined. Here we describe the synthesis of a chemically-releasable K^+ channel inhibitor that specifically targets KCNE peptides, which have assembled with K^+ channel subunits. Using this inhibitor in conjunction with electrical recordings, we count the number of KCNE peptides in functioning KCNQ1/KCNE1 K^+ channel complexes. Our results provide a definitive structural picture to interpret KCNE β-subunit modulation of voltage-gated K^+ channels and the inherited mutations that cause dysfunction. Given the wide assortment of specific ion channel inhibitors, our approach can be adapted to identify and enumerate the protein subunits in other multimeric ion channel complexes.

1748-Plat Structural Biophysical Basis for Modulation of Voltage-Gated Potassium Channels by KCNE1

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Human KCNE1, also known as MinK, is a 129 residue single span membrane protein that modulates voltage-gated potassium channels such as KCNQ1. For KCNQ1, binding of KCNE1 delays voltage-stimulated channel activation, while at the same time dramatically increasing the potassium ion conductance of the open state. The importance of this protein is highlighted by the fact that heritable mutations in this KCNE1 result in long QT syndrome, a sometimes fatal cardiac arrhythmia. Human KCNE1 was expressed in *E. coli* and purified into detergent micelles. Following studies involving microinjection of oocytes with recombinant KCNE1 followed by electrophysiological measurements, a suitable detergent was selected for structural studies. The structure of KCNE1 in detergent micelles was then determined using solution NMR spectroscopy. The experimental structure of KCNE1 was docked into models for the open and closed states of the KCNQ1 channel using the ROSETTA-Dock program. The results of these studies lead to a structurally-based model for how KCNE1 both delays channel activation and enhances channel conductance.

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1749-Plat An arrhythmia susceptibility gene in C. elegans

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kcne are evolutionarily conserved genes that encode accessory subunits of voltage-gated K^+ (Kv) channels. Missense mutations in *kcne1*, *kcne2* and *kcne3* are linked to congenital and acquired channelopathies in *Homo sapiens*. Here we show an unique example of conservation of *kcne* activities at genetic, physiological, functional and pathophysiological level in *Caenorhabditis elegans*. Thus, *mps-4* is the homologue of *kcne1* which operates in human heart and inner ear. Like its KCNE relatives, MPS-4 assembles with a Kv channel, EXP-2, to form a complex that controls pharyngeal muscle contractility. MPS-4 modulates EXP-2 function in a similar fashion as KCNE proteins endow human channels. When defective, MPS-4, can induce abnormal repolarization by mechanisms that resemble the way KCNE proteins are thought to provoke arrhythmia in human heart. Mutation of a conserved aspartate residue associated with human disease (MPS-4-D74N) alters the functional attributes of the *C. elegans* current. Taken together these data underscore a significant conservation of KCNE activities in different pumps.

This implies that *C. elegans* can develop into a system to study the molecular and genetic basis of KCNE-mediated muscle contractility and disease states.

1750-Plat KCNE2 Gene Structure and Direct Genomic Regulation by Estrogen

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K^+ channels are protein complexes made of pore-forming α-subunits and regulatory β-subunits, whose expression can be regulated by sex hormones. In particular, KCNE2 β-subunit forms complexes with a variety of K^+ channel α-subunits suggesting a broad spectrum of physiological roles, though its regulation by estrogen is unknown. To investigate whether *mouse* KCNE2 gene is regulated by estrogen, we experimentally identified KCNE2 transcription start sites and delineated its gene structure. We found a new exon (0) and mRNA with a retained intron. Analysis of 5′-flanking sequence to exon 0 revealed a promoter region with multiple vicinal start sites, a TATA sequence for transcriptional regulation and the presence of a quasi-perfect estrogen responsive element (ERE). Estrogen treatment stimulated KCNE2 promoter activity in a dose-dependent manner and the estrogen antagonist ICI 182,780 blocked estrogen stimulation. A direct genomic mechanism was demonstrated by: i) the lack of estrogen-responsiveness using a DNA-binding domain mutant estrogen receptor and by mutating the KCNE2 ERE, and ii) binding of estrogen receptor to the KCNE2-ERE. In vivo, KCNE2 transcripts were also upregulated in ovariectomized mice by estrogen treatment and the upregulation was prevented by ICI 182780. We conclude that estrogen upregulates KCNE2 transcription via a direct genomic regulatory mechanism finely tuning the cardiac excitability.

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1751-Plat The Role Of MiRP1 (*kcne2*) In Murine Ventricular Repolarization

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The MiRP1 ancillary subunit, encoded by the *KCNE2* gene, functionally co-assembles *in vitro* with a range of cardiac-expressed voltage-gated potassium (Kv) channel α subunits including hERG, KCNQ1 and Kv4.2. *KCNE2* mutations associate with inherited and acquired forms of human long QT syndrome, yet its relevance to cardiac physiology in any species is still questioned. Here, we disrupted the murine *kcne2* gene to begin to address this controversy. Neither echocardiography nor myocyte electron microscopy revealed abnormalities in *kcne2* (−/ −) mice. In transthoracic pacing studies, in contrast to *kcne2* (+/+) mice, *kcne2* (−/ −) mice were resistant to lengthening of the ventricular effective refractory period by 4-aminopyridine (4-AP). In whole-cell voltage-clamp studies of left ventricular myocytes, *kcne2* disruption reduced the density of two currents: $I_{to,f}$ (~25%) and $I_{K,slow}$ (~50%) (n = 14–23 cells per group; $p < 0.05$). In left ventricular apex and/or septal myocytes, *kcne2* disruption halved the 4-AP (50 μM) sensitive component of $I_{K,slow}$ current (generated by Kv1.5), and diminished by ~20 % the HpTx (300 nM) sensitive current ($I_{to,f,}$ - generated by Kv4 subunits). Currents sensitive to 25 mM TEA were unchanged. Western blots revealed MiRP1 expression in *kcne2* (+/+) but not (−/ −) murine ventricles. Quantitative Western blots from left ventricular membrane fractions revealed a ~50% reduction ($p < 0.05$) in Kv1.5 protein in *kcne2* (−/ −) mice compared to wild-type, with no significant changes in Kv1.4, Kv2.1, Kv4.2 or Kv4.3 protein expression (n = 3 x 10 hearts per group). Further, MiRP1 co-immunoprecipitated with Kv4.2 but not Kv4.3 or Kv1.4 in *kcne2* (+/+) murine ventricular membrane fractions. We conclude that MiRP1 contributes to murine ventricular repolarization by direct modulation of Kv4.2 ($I_{to,f}$). Also, in *kcne2* (−/ −) ventricles, Kv1.5 protein membrane expression is significantly reduced, diminishing the 4-AP sensitive component of $I_{K,slow}$.

Platform AI: Cryoelectron Microscopy & Reconstruction

1752-Plat Development Of Phase Plates Of Electron Microscopes For Their Biological Application

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To visualize transparent materials, transmission electron microscopy(TEM) traditionally has employed a phase contrast method, defocus phase contrast, that is commonly used in high resolution studies of inorganic materials(1). Unfortunately, a fundamental flaw in the application of this method to biological specimens is the difficulty of reconciling contrast and resolution. One approach for surmounting this problem would be to employ a phase contrast method that utilizes phase plates, similar to the method used to visualize transparent objects by light microscopy. Theoretically, electron microscopy is compatible with three different types of phase plates(2), the thin film, electrostatic, and magnetic. However, designing functional phase plates has been arduous process that has